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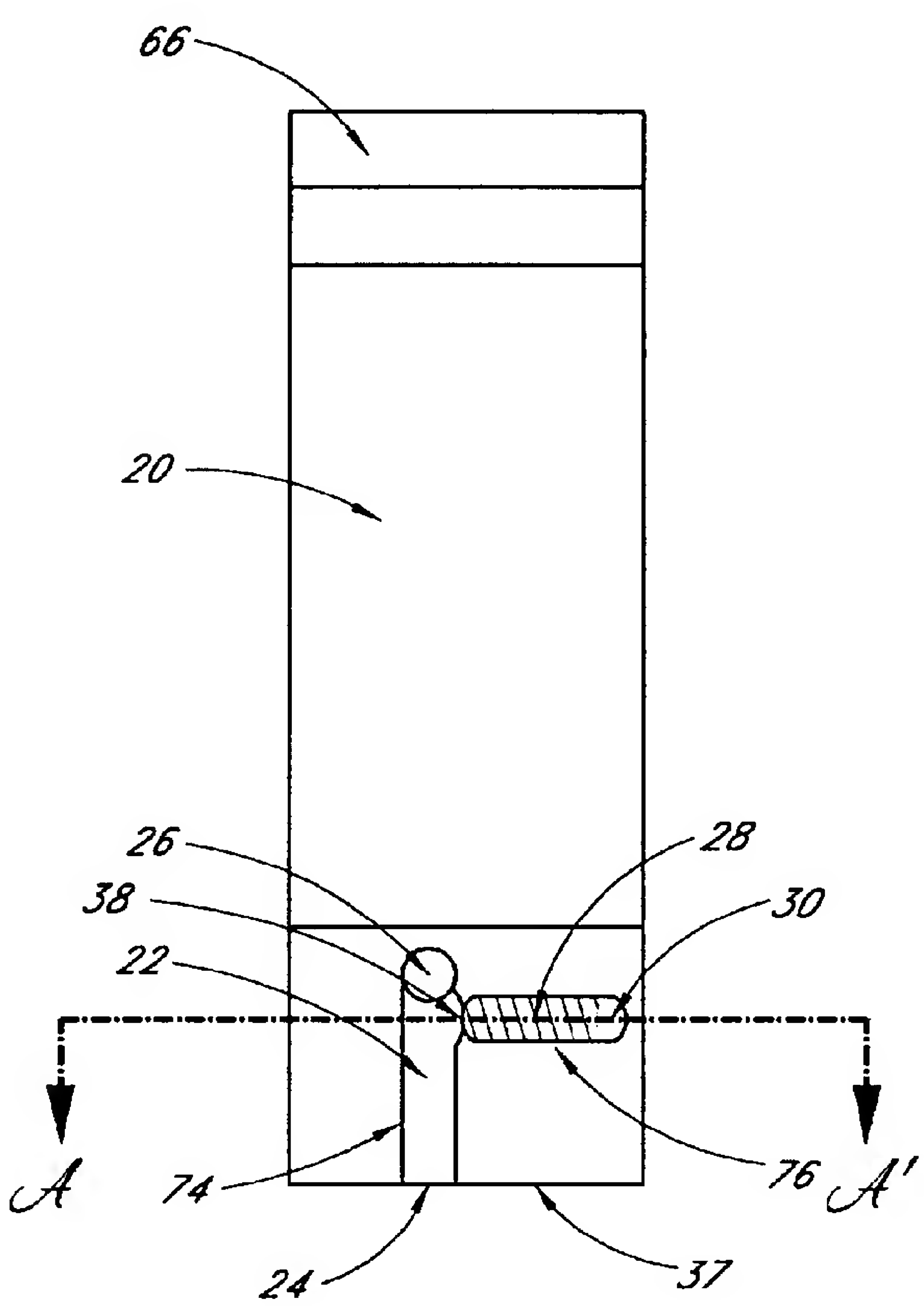
(54) Title: IMMUNOSENSOR



(57) Abstract: This invention describes a quantitative, inexpensive, disposable immunosensor 20 that requires no wash steps and thus generates no liquid waste. Moreover, in preferred embodiments of the sensor 20 no timing steps are required of the user, and the sensor 20 can be readily adapted to antigen-antibody interactions over a wide kinetic range.

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IMMUNOSENSOR

Field of the Invention

The present invention relates to a device and method for performing immunoassays. The device comprises a disposable immunosensor.

Background of the Invention

Biomedical sensors are used to report the presence and/or concentration of a wide variety of analytes. When the analyte is a protein, then the sensing element used is usually an antibody since the interaction of the antibody with the protein (antigen) is very specific. Such immunoassays usually fall into two categories: a "yes/no answer" obtained, e.g., by simple visual detection, or a concentration of the antigen determined by a quantitative method. Most of the quantitative methods involve expensive pieces of equipment such as scintillation counters (for monitoring radioactivity), spectrophotometers, spectrofluorimeters (*see, e.g.,* U.S. 5,156,972), surface plasmon resonance instruments (*see, e.g.,* U.S. 5,965,456), and the like. It would therefore be advantageous to develop a quantitative immunoassay that is both inexpensive and simple enough to use to be suitable for home or field use.

Conventional immunoassays are classified into two categories: competition assay and sandwich assay. In a competition assay, the antigen in the test sample is mixed with an antigen-probe complex and the mixture then competes for binding to the antibody. The probe may be an enzyme, a fluorophore or a chromophore. Secondly, in a sandwich immunoassay, the antigen in the test sample binds to the antibody and then a second antibody-probe complex binds to the antigen. In these prior art assay methods, one or more washing steps are usually required. The washing steps introduce complexity into the assay procedure and can generate biohazardous liquid waste. It would therefore be advantageous to develop a device for performing an immunoassay that does not require any washing steps. Of necessity, such a device would be designed to be a single use disposable device.

Summary of the Invention

A quantitative, inexpensive, disposable immunosensor is provided that requires no wash steps and thus generates no liquid waste. Moreover, in preferred embodiments of the sensor, no timing steps are required of the user, and the sensor can be readily adapted to antigen-antibody interactions over a wide kinetic range.

In one embodiment, a disposable device is provided for use in detecting a target antigen in a fluid sample having a pH, the device including a reaction chamber having an internal surface, a proximal end, and a distal end; an immobilized antibody fixed within the reaction chamber, the antibody being capable of binding to the target antigen; a reporter complex present within the reaction chamber, the complex including a probe, the reporter complex being capable of mixing with the sample; a detection chamber having a wall, an internal surface, a distal end and a proximal end; a sample ingress at the distal end of the reaction chamber; and a sample passageway between the distal end of the reaction chamber and the proximal end of the detection chamber.

In one aspect of this embodiment, an agent contained within the reaction chamber and capable of preventing non-specific binding of proteins to the reaction chamber internal surface is included. The agent may be selected from the group consisting of a surfactant and a blocking protein, for example, bovine serum albumin.

In another aspect of this embodiment, the reporter complex further includes a second antigen capable of competing with the target antigen for binding to the immobilized antibody, or a second antibody capable of binding to the target antigen.

In another aspect of this embodiment, the probe is selected from the group consisting of chromophores and fluorophores. The probe may include an enzyme, such as glucose oxidase or glucose dehydrogenase. An enzyme substrate may also be included, for example, an oxidizable substrate such as galactose, acetic acid, or glucose.

In another aspect of this embodiment, the detection chamber further includes a mediator. The mediator may include dichlorophenolindophenol, complexes between transition metals and nitrogen-containing heteroatomic species, or ferricyanide.

In another aspect of this embodiment, the device further includes a buffer capable of adjusting the pH of the sample, such as one including phosphate or citrate.

In another aspect of this embodiment, the immobilized antibody and/or the reporter complex is supported on a reaction chamber interior surface. The reporter complex may be separated from the immobilized antibody by less than about 1 millimeter.

In another aspect of this embodiment, the device further includes a stabilizer that stabilizes one or more of the antigen, the enzyme, and the antibody.

In another aspect of this embodiment, the enzyme substrate is supported on a detection chamber interior surface.

In another aspect of this embodiment, the device further includes a support material. The support material may be contained within the detection chamber, and one or more substances such as an enzyme substrate, a mediator, and a buffer may be supported on or contained within the support material. The support material may also be contained within the reaction chamber, and one or more substances such as the immobilized antibody, the reporter complex, and an agent capable of preventing non-specific binding of proteins to the reaction chamber internal surface may be supported on or contained within the support material. The support material may include a mesh or fibrous filling material including a polymer selected from the group consisting of polyolefin, polyester, nylon, cellulose, polystyrene, polycarbonate, polysulfone, and mixtures thereof; a porous material such as a macroporous membrane including a polymeric material selected from the group consisting of polysulfone, polyvinylidene difluoride, nylon, cellulose acetate, polymethacrylate, polyacrylate, and mixtures thereof; or a sintered powder.

In another aspect of this embodiment, the detection chamber includes at least two electrodes. The electrodes may include a material selected from the group consisting of palladium, platinum, gold, iridium, carbon, carbon mixed with binder, indium oxide, tin oxide, and mixtures thereof.

In another aspect of this embodiment, the detection chamber wall is transparent to a radiation emitted or absorbed by the probe, the radiation being indicative of the presence or absence of the reporter complex in the detection chamber.

In another aspect of this embodiment, a detector capable of detecting a condition wherein the reaction chamber is substantially filled is included. A piercing means capable of forming a detection chamber vent in the distal end of the detection chamber may also be included. A reaction chamber vent at the distal end of the reaction chamber may be included as well.

In a second embodiment, a method of manufacture of a disposable device for use in detecting a target antigen in a fluid sample having a pH is provided, the method including the steps of forming a first aperture extending through a first sheet of material having a proximal end and a distal end, the first aperture defining a reaction chamber side wall, a detection chamber side wall and a first sample passageway between the reaction chamber distal end and the detection chamber proximal end; mounting a first layer to a first side of the first sheet and extending over the aperture to define a first reaction chamber end wall and a first detection chamber end wall; mounting a second layer to a second side of the first sheet and extending over the aperture to define a second reaction chamber end wall and a second detection chamber end wall in substantial overlying registration with the first layer, whereby the sheet and layers form a strip having a plurality of exterior surfaces; forming a second passageway extending through an exterior surface of the strip and into the reaction chamber at the reaction chamber distal end, the second passageway defining a reaction chamber vent; forming a third passageway extending through the an exterior surface of the strip and into the reaction chamber at the reaction chamber proximal end, the third passageway defining a sample ingress; immobilizing an antibody within the reaction chamber; and placing a reporter complex in the reaction chamber, the complex including a probe.

In one aspect of this embodiment, the aperture extends through the proximal end of the first sheet to form the third passageway.

In another aspect of this embodiment, the first sheet, the first layer and the second layer include an electrically resistive material, the first layer includes a first electrode wherein the first electrode faces the first side of the first sheet, and the second layer includes a second electrode wherein the second electrode faces the second side of the sheet. At least one of the electrodes may include a material selected from the group consisting of palladium, platinum, gold, iridium, carbon, carbon mixed with binder, indium oxide, tin oxide, and mixtures thereof. The first electrode may substantially cover the first detection chamber end wall and the second electrode substantially covers the second detection chamber end wall. At least one of the electrodes may be a sputter coated metal deposit. The second electrode may be mounted in opposing relationship a distance of less than about 500 microns from the first electrode; less than about 150 microns from the first electrode; or less than about 150 microns and greater than about 50 microns from the first electrode.

In another aspect of this embodiment, the layers are adhered to the sheet, for example, by an adhesive such as a heat activated adhesive, pressure sensitive adhesive, heat cured adhesive, chemically cured adhesive, hot melt adhesive, and hot flow adhesive. At least the sheet, or one of the layers may include a polymeric material such as polyester, polystyrene, polycarbonate, polyolefin, and mixtures thereof, or polyethylene terephthalate. At least one of

the layers may be transparent to a wavelength of radiation including infrared radiation, visible light, and ultraviolet radiation.

In another aspect of this embodiment, the method further includes providing an enzyme substrate and a mediator, wherein the enzyme substrate and the mediator are contained within the detection chamber, wherein the probe is an enzyme, and wherein the mediator is capable of mediating a reaction between the enzyme and the electrode, to indicate the occurrence of an electrochemical reaction.

In another aspect of this embodiment, the method further includes the step of providing a buffer, wherein the buffer is capable of adjusting the pH of the sample.

In a third embodiment, a method of manufacture of a disposable device for use in detecting a target antigen in a fluid sample having a pH is provided, the method including forming a first aperture extending through a first sheet of electrically resistive material having a proximal end and a distal end, the first aperture having a first aperture reaction chamber part and a first aperture detection chamber part and defining a first portion of a reaction chamber side wall, a detection chamber side wall and a sample passageway between the reaction chamber distal end and the detection chamber proximal end; forming a second aperture extending through a second sheet of electrically resistive material having a proximal end and a distal end, the second aperture defining a second portion of the reaction chamber side wall; forming a third aperture extending through a third sheet of electrically resistive material having a proximal end and a distal end, the third aperture defining a third portion of the reaction chamber side wall; mounting a first side of the second sheet to a first side of the first sheet, the second sheet extending over the first aperture detection chamber part whereby to define a first detection chamber end wall, the second portion of the reaction chamber side wall in substantial registration with the first portion of the reaction chamber side wall; mounting a first side of the third sheet to a second side of the first sheet, the third sheet extending over the first aperture detection chamber part whereby to define a second detection chamber end wall, the third portion of the reaction chamber side wall in substantial registration with the first portion of the reaction chamber side wall; mounting a first layer to a second side of the second sheet and extending over the second aperture to define a first reaction chamber end wall; mounting a second layer to a second side of the third sheet and extending over the third aperture to define a second reaction chamber end wall in substantial overlying registration with the first thin layer, whereby the sheets and layers form a strip having a plurality of exterior surfaces; forming a second passageway extending through the outside of the strip and into the reaction chamber at the reaction chamber distal end, the second passageway defining a reaction chamber vent; forming a third passageway extending through the outside of the strip and into the reaction chamber at the reaction chamber proximal end, the third passageway defining a sample ingress; immobilizing an antibody within the reaction chamber; and placing a reporter complex in the reaction chamber, the reporter complex including a probe.

In a fourth embodiment, a method for determining a presence or an absence of a target antigen in a fluid sample is provided, the method including providing a disposable device including a reaction chamber having an internal surface, a proximal end, and a distal end, an immobilized antibody fixed within the reaction chamber, the antibody being capable of binding to the target antigen, a reporter complex present within the reaction chamber, the complex

including a probe, the reporter complex being capable of mixing with the sample, a detection chamber having a wall, an internal surface, a distal end and a proximal end, a sample ingress at the distal end of the reaction chamber, and a sample passageway between the distal end of the reaction chamber and the proximal end of the detection chamber, wherein the reporter complex further includes a second antigen capable of competing with the target antigen for binding to the immobilized antibody; contacting a fluid sample with the sample ingress; substantially filling the reaction chamber with the fluid sample by allowing the sample to flow from the sample ingress toward the reaction chamber; allowing a predetermined time to lapse, the time being sufficient for substantially all reporter complex to bind to the immobilized antibody in the absence of antigen in the sample; substantially filling the detection chamber with the fluid sample by allowing the sample to flow from the reaction chamber through the sample passageway toward the detection chamber; detecting a presence or an absence of the antigen-probe complex within the detection chamber, the presence or absence of the antigen-probe complex being indicative of a presence or an absence of the antigen in the sample.

In one aspect of this embodiment, then method further includes piercing the wall of the detection chamber so as to form a detection chamber vent at the distal end of the detection chamber, the piercing step immediately following the lapse of the predetermined time.

In a fifth embodiment, a method of manufacture of a disposable device for use in detecting a target antigen in a fluid sample having a pH is provided, the device having a plurality of exterior surfaces, the method including forming a first aperture extending through a first sheet of electrically resistive material, the first aperture having a detection chamber part and defining a detection chamber side wall, the detection chamber having a proximal end and a distal end; mounting a first layer to a first side of the first sheet and extending over the aperture to define a first detection chamber end wall; mounting a second layer to a second side of the first sheet and extending over the aperture to define a second detection chamber end wall in substantial overlying registration with the first layer, whereby the sheet and layers form a strip; forming a second aperture extending through the strip, the strip having a proximal end and a distal end, the second aperture having a reaction chamber part, the reaction chamber having a distal end and a proximal end, and the second aperture defining a reaction chamber side wall and a sample passageway between the reaction chamber distal end and the detection chamber proximal end; mounting a first side of a third layer to a first side of the strip, the third extending over the second aperture reaction chamber part to define a first reaction chamber end wall; mounting a first side of a fourth layer to a second side of the strip, the fourth layer extending over the second aperture reaction chamber part whereby to define a second reaction chamber end wall in substantial registration with the first reaction chamber end wall; forming a third aperture extending through a surface of the device and into the reaction chamber at the reaction chamber distal end, the third aperture defining a reaction chamber vent; forming a fourth aperture extending through a surface of the device and into the reaction chamber at the reaction chamber proximal end, the fourth aperture defining a sample ingress; immobilizing an antibody within the reaction chamber; and placing a reporter complex in the reaction chamber, the reporter complex including a probe.

In one aspect of this embodiment, the first sheet, the first layer and the second layer include an electrically resistive material, the first layer includes a first electrode wherein the first electrode faces the first side of the first sheet, and the second layer includes a second electrode wherein the second electrode faces the second side of the sheet.

Brief Description of the Drawings

FIG. 1 shows a top view (not to scale) of an immunosensor incorporating an electrochemical cell.

FIG. 2 shows a cross-sectional view (not to scale) along line A-A' of an embodiment of the immunosensor of Figure 1.

Detailed Description of the Preferred Embodiments

The following description and examples illustrate a preferred embodiment of the present invention in detail. Those of skill in the art will recognize that there are numerous variations and modifications of this invention that are encompassed by its scope. Accordingly, the description of a preferred embodiment should not be deemed to limit the scope of the present invention.

Disclosed is a single step, no-wash immunosensor. The sensor is a single use, disposable device that utilizes two adjacent chambers, a reaction chamber and a detection chamber. In the reaction chamber, the antigen-antibody reactions take place and in the detection chamber the results of those reactions are detected and the presence or absence of antigen in the sample is inferred.

Any suitable detection method can be utilized. Suitable detection methods include, e.g., visual detection wherein the development of a color is observed, or spectroscopic detection wherein reflected or transmitted light is used to measure changes in light absorbance. In a preferred embodiment, the detection method is electrochemical wherein the electrical current or potential generated indirectly by the products of antigen/antibody reactions is measured.

Methods and devices for obtaining electrochemical measurements of fluid samples are discussed further in copending U.S. patent application no 09/615,691, filed on July 14, 2000, entitled "ANTIOXIDANT SENSOR," copending U.S. patent application no 09/616,512, filed on July 14, 2000, entitled "HEMOGLOBIN SENSOR," and copending U.S. patent application no 09/616,556, filed on July 14, 2000, entitled "ELECTROCHEMICAL METHOD FOR MEASURING CHEMICAL REACTION RATES," each of which is incorporated herein by reference in its entirety.

The timing of the various test stages, i.e., the reaction stage and the detection stage may be done manually. Alternatively, timing may be done automatically in response to a trigger signal generated when the reaction chamber is filled.

An embodiment of the sensor suitable for use with electrochemical detection is illustrated in FIGS. 1 and 2. FIG. 1 is a top view of the sensor strip and FIG. 2 is a cross-sectional view, showing details of the reaction chamber and the detection chamber.

The Sensor

The immunosensors of the preferred embodiments may be prepared using well-known thin layer device fabrication techniques as are used in preparing electrochemical glucose sensing devices (see, e.g., U.S. 5,942,102, incorporated herein by reference in its entirety). Such techniques, with certain modifications, are also used to prepare immunosensors utilizing non-electrochemical detection methods.

In a preferred embodiment of the immunosensor, illustrated in Figures 1 and 2, the detection chamber **28** comprises an electrochemical cell **28**. The reaction chamber **22** and detection chamber **28** are prepared by first forming an aperture extending through a sheet of electrically resistive material **36**. The aperture is shaped such that it defines a sidewall of both the reaction chamber **22** and the detection chamber **28**, as well as the sample passageway **38** between the two chambers **22** and **28**. By extending the aperture from the proximal end **24** of the reaction chamber **22** through to the edge of the sheet **37**, the sample ingress **24** is also formed. In one embodiment, the thickness of the sheet **36** defines the entire height of the reaction chamber **22** and detection chamber **28**, which are the same. In another embodiment, the height of the reaction chamber **22** is greater than that of the detection chamber **28**. A reaction chamber **22** of greater height than the detection chamber **28** is prepared by layering multiple sheets **32**, **34**, and **36** together. The middle sheet **36** of the layer has an aperture defining the sidewalls **74** and **76** of both the reaction chamber **22** and detection chamber **28** as described above. This middle layer **36** is then sandwiched between two or more additional layers **32** and **34**, the additional layers **32** and **34** having an aperture defining the side wall **74** of the reaction chamber **22** only, the layers **32** and **34** thereby defining end walls **60** and **62** of the detection chamber **28**. In this embodiment, the end walls **60** and **62** of the detection chamber comprise electrodes **52** and **54**, which may be prepared as described below.

After the sidewalls **74** and **76** of the reaction chamber **22** and detection chamber **28** are formed, a first thin electrode layer **52** is then mounted on one side **70** of the sheet of electrically resistive material **36**, extending over the aperture forming the detection chamber **28** and forming an end wall **60**. The layer **52** may be adhered to the sheet **36**, e.g., by means of an adhesive. Suitable adhesives include, for example, heat activated adhesives, pressure sensitive adhesives, heat cured adhesives, chemically cured adhesives, hot melt adhesives, hot flow adhesives, and the like. The electrode layer **52** is prepared by coating (e.g., by sputter coating) a sheet of electrically resistive material **32** with a suitable metal, for example, platinum, palladium, carbon, indium oxide, tin oxide, mixed indium/tin oxides, gold, silver, iridium, mixtures thereof, and the like. Materials suitable for use as electrodes **52** and **54** are compatible with the reagents present in the sensor **20**, i.e., they will not react chemically with reagents.

A second thin electrode layer **54** is then mounted on the opposite side **71** of the electrically resistive material **36**, also extending over the aperture forming the detection chamber **28**, so as to form a second end wall **62**. In a preferred embodiment, the electrode layers **52** and **54** are mounted in opposing relationship at a distance of less than about 500 microns, more preferably less than 150 microns, and most preferably between 50 and 150 microns. If a sample ingress **24** has not already been formed, then one is provided, e.g., by forming a notch in the edge **37** of the device **20** that intersects the proximal end **23** of the reaction chamber **22**.

The electrode layers **52** and **54** are provided with connection means allowing the sensor **20** to be placed in a measuring circuit. At least one of the electrodes **52** or **54** in the cell **28** is a sensing electrode, i.e., an electrode sensitive to the amount of reduced redox agent in the antioxidant case or oxidized redox agent in the oxidant case. In the case of a potentiometric sensor **20** wherein the potential of the sensing electrode **52** or **54** is indicative of the level of analyte present, a second electrode **54** or **52**, acting as reference electrode is present which acts to provide a reference potential. In the case of an amperometric sensor **20** wherein the sensing electrode current is indicative of the level of analyte in the sample, at least one other electrode **54** or **52** is present which functions as a counter electrode to complete the electrical circuit. This second electrode **54** or **52** may also function as a reference electrode. Alternatively, a separate electrode (not shown) may perform the function of a reference electrode.

If the immunosensor **20** is operated as an electrochemical cell **28**, then the sheets **32**, **34**, and **36** containing the apertures defining the reaction chamber **22** and/or detection chamber **28** should comprise electrically resistive materials. Suitable electrically resistive materials include, for example, polyesters, polystyrenes, polycarbonates, polyolefins, mixtures thereof, and the like. A preferred polyester is polyethylene terephthalate. If the immunosensor **20** is operated using a detection method other than an electrochemical detection method, then the materials need not be electrically resistive. However, the polymeric materials described above are preferred for use in constructing the immunosensors of a preferred embodiment because of their ease of processing, low cost, and lack of reactivity to reagents and samples. In the case of a detection method involving absorbance, transmission, or emission of light of a particular frequency, then the end walls **60** and/or **62** and layers **32** and **46** and/or layers **34** and **42** above the end walls of the detection chamber **28** should be transparent to that light frequency.

Reagents for use in the cell **28**, e.g., immobilized antibody, probe-linked antigen, buffer, mediator, and the like, may be supported on the walls **40**, **48**, and/or **74** of the reaction chamber **22** or on the walls **60**, **62**, and/or **76** of the detection chamber **28**, on an independent support contained within chambers, within a matrix, or may be self supporting. If the reagents are to be supported on the chamber walls or electrodes **52** and **54**, the chemicals may be applied by use of printing techniques well known in the art, e.g., ink jet printing, screen printing, lithography, and the like. In a preferred embodiment, a solution containing the reagent is applied to a surface within a chamber and allowed to dry.

Rather than immobilize or dry the antibodies **44**, the probe-linked antigen **50**, or other chemicals onto the surfaces **40**, **48**, **60**, **62**, **74**, and/or **76** of the reaction chamber **22** or detection chamber **28**, it may be advantageous to support them on or contain them within one or more independent supports which are then placed into a chamber. Suitable independent supports include, but are not limited to, mesh materials, nonwoven sheet materials, fibrous filling materials, macroporous membranes, or sintered powders. The advantages of independent supports include an increased surface area, thus allowing more antibody and probe-linked antigen to be included in the reaction chamber **28**, if desired. In such an embodiment, the antibody is immobilized on one piece of porous material and placed in the first reaction chamber and the probe-linked antigen is dried onto a second piece of porous material, which is then placed into the reaction chamber. Alternatively, either the antibody or the probe-linked antigen is incorporated onto the

porous material and the other component supported on the reaction chamber wall as described above. In yet another embodiment, the walls of the reaction chamber themselves are porous, with the antibody and/or probe-linked antigen incorporated in them. In this embodiment, the liquid is able to wick into the porous wall, but not leak out of the defined area. This is accomplished by using a macroporous membrane to form the reaction chamber wall and compressing the membrane around the reaction chamber to prevent leakage of sample out of the desired area.

Suitable independent supports such as mesh materials, nonwoven sheet materials, and fibrous fill materials include, polyolefins, polyesters, nylons, cellulose, polystyrenes, polycarbonates, polysulfones, mixtures thereof, and the like. Suitable macroporous membranes may be prepared from polymeric materials including polysulfones, polyvinylidene difluorides, nylons, cellulose acetates, polymethacrylates, polyacrylates, mixtures thereof, and the like.

The protein or antibody may be contained within a matrix, e.g., polyvinyl acetate. By varying the solubility characteristics of the matrix in the sample, controlled release of the protein or antibody into the sample may be achieved.

In all cases, the materials used within the sensor are in a form amenable to mass production, and the cells themselves are designed to be able to be used for a single experiment then disposed of.

A preferred embodiment of an immunosensor that is fabricated as described above is illustrated in Figures 1 and 2. In this preferred embodiment, the layers **32** and **34** are substrates coated with electrically conductive material **52** and **54**. The electrically conductive material **52** or **54** on the surface **60** or **62** facing the detection chamber **28** and an adhesive layer (not shown) coated on the surface **33** or **35** facing layer **42** or **46**, respectively.

<u>Using the Sensor to Determine the Presence or Absence of an Antigen</u>

In a preferred embodiment, the sensor **20** is an electrochemical cell **28** utilizing an enzyme, e.g., glucose oxidase or glucose dehydrogenase, as the probe, as illustrated in Figure 1, a top view of such a sensor **20**, and Figure 2, a cross section of the sensor through line A-A′. The presence or absence of an analyte is inferred in this embodiment as follows.

The user first introduces sample into the reaction chamber **22** of the sensor **20** through the sample ingress **24**. The sample is drawn into the reaction chamber **22** under the influence of capillary or wicking action. During filling the reaction chamber vent **26** is open to the atmosphere, thus allowing air displaced by the sample to escape. Sample will be drawn into the reaction chamber **22** until it is filled up to the reaction chamber vent **26**, whereupon filling will stop. The volume of the reaction chamber **22** is chosen so as to be at least equal to and preferably larger than the volume of the detection chamber **28**.

The dashed circle in Figure 1 denotes an aperture **30** piercing layers **32**, **34**, and **36** but not layers **42** and **46**, the aperture in layer **34** opening into the detection chamber **28**. Since layers **42** and **46** are not pierced initially, the only opening to the atmosphere of the detection chamber **28** is the sample passageway **38** opening into the reaction chamber **22**. Thus, when the reaction chamber **22** fills with sample, it blocks the sample passageway **38** to the detection chamber **28**. This traps air in the detection chamber **28** and substantially prevents it from filling with sample. A small amount of sample will enter the detection chamber **28** during the time between when the sample first

contacts the opening **38** to the detection chamber **28** and when it contacts the far side of the opening **38**. However, once the sample has wet totally across the opening **38** to the detection chamber **28**, no more filling of the detection chamber **28** will take place.

The internal surface **40** of the substrate **42**, which forms the base of the reaction chamber **22**, is coated with antibodies **44** to the antigen to be detected. The antibodies **44** are adsorbed or otherwise immobilized on the surface **40** of the substrate **42** such that they are not removed from the substrate **42** during a test. Optionally, after application of the antibodies **44** to the internal surface **40** of the substrate **42**, an agent designed to prevent non-specific binding of proteins to this surface can be applied (not shown). An example of such an agent well known in the art is bovine serum albumin (BSA). A nonionic surfactant may also be used as such an agent, e.g., Triton X100™ manufactured by Rohm & Haas of Philadelphia, Pennsylvania, or Tween™ manufactured by ICI Americas of Wilmington, Delaware. The nonionic surfactant selected does not denature proteins. The coating **44** on the internal surface **40** of the substrate **42** is in the dry state when ready to be used in a test.

Another substrate **46** defines the top surface **48** of the reaction chamber **22**. On the internal surface **48** of the substrate **46** are coated enzymes linked to the antigen **50** to be detected. Examples of suitable enzymes include, but are not limited to, glucose oxidase and glucose dehydrogenase. The enzyme-linked antigen **50** is dried onto the internal surface **48** of the substrate **66** in such a way that it can be liberated into the sample when this surface **48** is wet by the sample. The internal surface **48** of the substrate **46** and the method for coating on the enzyme-linked antigen **50** are therefore chosen such that only a weak bond between the enzyme-linked antigen **50** and the internal surface **48** of the substrate **46** exists. The rate of dissolution of the enzyme-linked antigen **50** from the surface **48** is chosen such that little dissolution has occurred during the time taken for the sample to fill the reaction chamber **22**. In this manner, the enzyme-linked antigen **50** will be evenly distributed throughout the area of the reaction chamber **22** after filling.

The relative amounts of enzyme-linked antigen **50** and antibody **44** are chosen such that there is a slight excess of antibody **44** over enzyme-linked antigen **50**. In this context, a slight excess is defined to be such that the excess is small when compared to the number of antigen molecules to be detected in the sample.

Thus, when sample fills the reaction chamber **22** the enzyme-linked antigen **50** enters and mixes with the sample. Sufficient time is then allowed for the enzyme-linked antigen **50** to come into contact with the antibodies **44**. Since there is an excess of antibodies **44**, if no antigen is present in the sample then substantially all of the enzyme-linked antigen **50** will bind to the antibodies **44** and so be effectively immobilized. If antigen is present in the sample, the antigen, being smaller than the enzyme-linked antigen **50** and already present throughout the volume of the sample, will contact and bind to the antibodies **44** before the enzyme-linked antigen **50** contacts the antibodies **44**. The antibodies **44** will therefore be blocked and prevented from binding to the enzyme-linked antigen **50**. So if antigen is initially present in the sample then, at the end of the reaction step, enzyme-linked antigen **50** will remain mobile in the sample. If no antigen is initially present in the sample, the enzyme-linked antigen **50** will be immobilized on the internal surface **48** of the substrate **46** at the end of the reaction step.

The end of the reaction step is a predetermined time after the sample is introduced into the reaction chamber **22**. The predetermined time is set such that there is sufficient time for substantially all of the enzyme-linked antigen **50** to bind to the antibodies **44** under the test conditions when no antigen is initially present in the sample.

The time that the sample is introduced into the reaction chamber **22** can be indicated by the user, for example, by depressing a button on a meter connected to the sensor **20**. This action is used to trigger a timing device. In the case of visual detection, no meter device is necessary. In such an embodiment, the user manually times the reaction period.

In the case where electrochemical detection is used to detect the result of the antibody/antigen reactions, the indication that sample has been introduced into the reaction chamber **22** can be automated. As described above, when sample fills the reaction chamber **22**, a small portion of the detection chamber **28** at its opening **38** into the reaction chamber **22** will be wet by sample. If electrochemical detection is employed then at least two electrodes **52** and **54** will be present in the detection chamber **28**. If these electrodes **52** and **54** are placed in the detection chamber **28**, such that at least a portion of each electrode **52** and **54** is contacted by the sample during the filling of the reaction chamber **22**, the presence of the sample will bridge the electrodes **52** and **54** and create an electrical signal which can be used to trigger the timing device.

A predetermined time after the timing device has been triggered, either by the user or automatically, the antibody/antigen reaction phase of the test is deemed to be completed. When the antibody/antigen phase of the test is completed, the vent **56** to the atmosphere is opened. For example, a solenoid activated needle in the meter may be used to pierce layer **42** or layer **46** or both layers **42** and **46**, thus opening the distal end **58** of the detection chamber **28** to the atmosphere. The piercing can be automatically performed by the meter, as in the example above, or manually by the user in the case of visual detection wherein no meter may be used, e.g., the user inserts a needle through the layers **42** and **46**, thereby forming the vent **56**.

The opening of the vent **56** to the atmosphere allows the air trapped in the detection chamber **28** to escape, thereby allowing the detection chamber **28** to be filled with reacted sample from the reaction chamber **22**. The reacted sample will be drawn into the detection chamber **28** due to increased capillary force in the detection chamber **28** compared to that present in the reaction chamber **22**. In a preferred embodiment, the increased capillary force is provided by suitably coating the surfaces **60** and **62** of the detection chamber **28** or, more preferably, by choosing the capillary distance for the detection chamber **28** to be smaller than that of the reaction chamber **22**. In this embodiment, the capillary distance is defined to be the smallest dimension of the chamber.

Optionally disposed in the detection chamber **28** are dried reagents **64** comprising an enzyme substrate and a mediator, capable of reacting with the enzyme part of the enzyme-linked antigen **50** to produce a detectable signal. The enzyme substrate and mediator, if present, are to be of sufficient amount such that the rate of reaction of any enzyme present with the enzyme substrate **64** is determined by the amount of enzyme present. For instance, if the enzyme were glucose oxidase or glucose dehydrogenase, a suitable enzyme mediator **64** and glucose (if not already present in the sample) would be disposed into the detection chamber **28**. Buffer may also be included to help adjust

the pH of the sample in the detection chamber **28**. In an embodiment wherein an electrochemical detection system is used, ferricyanide is a suitable mediator. Other suitable mediators include dichlorophenolindophenol and complexes between transition metals and nitrogen-containing heteroatomic species. The glucose, mediator and buffer reagents **64** are present in sufficient quantities such that the rate of reaction of the enzyme with the enzyme substrate **64** is limited by the concentration of the enzyme present.

When the detection chamber **28** is filled, the reagents **64** dissolve into the sample. The enzyme component of the reagents **64** reacts with the glucose and the mediator to produce reduced mediator. This reduced mediator is electrochemically oxidized at an electrode **52** or **54** acting as an anode in the detection chamber **28** to produce an electrical current. In one embodiment, the rate of change of this current with time is used as an indicator of the presence and amount of enzyme that is present in the reacted sample. If the rate of change of current is less than a predetermined threshold value, then it is indicative of no significant amount of enzyme-linked antigen **50** present in the reacted sample, indicating the lack of antigen present in the original sample. If the rate of change of current is higher than the threshold rate, it indicates that enzyme-linked antigen **50** is present in the reacted sample, and thus antigen is also present in the sample initially. In one embodiment, the rate of change of the current is used to give a measure of the relative amount of antigen initially present in the sample.

In a preferred embodiment of the electrochemical detection system, the electrodes **52** and **54** in the detection chamber **28** are formed as electrically conductive layers coated onto the internal surfaces **60** and **62** of substrates **32** and **34**, e.g., by sputtering as disclosed in WO97/18464. These conductive layers **52** and **54** are of materials that do not react chemically with reagent present and are useful as electrodes **52** and **54** at the potential of choice. Examples of suitable materials include, but are not limited to, palladium, platinum, gold, iridium, carbon, carbon mixed with a binder, indium oxide, tin oxide, and mixed oxides of indium and tin.

In this embodiment, an inert, electrically insulating layer **36** separates the electrode-bearing substrates **32** and **34**. Preferably, insulating layer **36** functions to keep layers **32** and **34** at a predetermined separation. Provided this separation is small enough, e.g., less than 500 micron and more preferably from 50 to 150 microns, the current flowing between the electrodes **52** and **54** will be directly proportional to the concentration of reduced mediator after a suitably short time relative to the detection time employed. In this embodiment, the rate of current rise is directly related to the rate of the enzyme reaction and therefore the amount of enzyme present.

In Figure 1, a connection end **66** is shown. The electrodes **54** and **56** in the detection chamber **28** can be placed in electrical connection with a meter (not shown) through the connection end **66**. The connection means (not shown) are in electrical connection with the electrodes **54** and **56** in the detection chamber **28** via conducting tracks (not shown). In the preferred embodiment illustrated in Figure 1, these conducting tracks consist of extensions of the films of conductor **52** and **54** coated onto the internal surfaces of **32** and **34**. The meter in connection with the connection area **66** is capable of applying a potential between the electrodes **52** and **54** in the detection chamber **28**, analyzing the electrical signals generated, displaying a response and optionally storing the response in memory.

In other embodiments utilizing electrochemical detection, stripes of conducting material on one or both internal faces of the detection chamber are used, with the provision that at least two electrodes are present, i.e., a sensing electrode and a counter/reference electrode. Optionally, a third electrode, serving as a separate reference electrode, is included.

In the case of an embodiment wherein visual detection or reflectance spectroscopy is the detection method used, at least the layers **32** and **46** or layers **34** and **42** are transparent to the wavelength of radiation that is to be observed. In the case of visual detection, a simple color change in the detection chamber **28** is observed. In the case of reflectance spectroscopy, detection radiation is shone through layers **32** and **46** or layers **34** and **42**, and radiation reflected from the solution in the detection chamber **28** is analyzed. In the case of transmission spectroscopy used as the detection method, at least layers **32**, **46**, **34**, and **42** are transparent to radiation at the wavelength of choice. Radiation is shone through the sample in the detection chamber **28** and the attenuation of the beam is measured.

In a preferred embodiment of a method of constructing the sensor, layer **36** comprises a substrate with a layer of adhesive (not shown) coated on its upper surface **70** and lower surface **72**. Examples of materials suitable for the substrate of layer **36** include polyester, polystyrene, polycarbonate, polyolefins, and, preferably, polyethylene terephthalate. Examples of suitable adhesives are pressure sensitive adhesives, heat and chemically curing adhesives and hot melt and hot flow adhesives.

Use of Melitten as a Probe

Conventional ELISAs link an antigen to an enzyme. However, it is also possible to link the antigen to melittin, a polypeptide found in bee venom. In this embodiment, a probe-linked antigen comprising an antigen-melittin complex can be dried on a wall of the reaction chamber, as described above. The detection chamber can contain a mediator comprising ferrocyanide in liposomes or lipid vesicles. If the antigen-melittin complex reaches the liposomes, they will burst and release the ferrocyanide. This leads to a rapid amplification of the signal, i.e., a small amount of free antigen competes with the antigen-melittin complex for binding sites on the antibodies and results in a large concentration of ferrocyanide.

Use of Horse Radish Peroxidase and Alkaline Phosphatase in Electrochemical Assays

Conventional ELISAs use horse radish peroxidase (HRP) or alkaline phosphatase (AP) as the enzymes in a colorimetric assay. However, substrates have been developed which allow both these enzymes to be used in an electrochemical assay.

In this embodiment, AP can be used with p-aminophenyl phosphate and HRP can be used with tetrathiafulvalene.

Obtaining Electrochemical Measurements Using The Immunosensor

In certain embodiments, information relating to the rate of a chemical reaction that yields at least one electroactive product can be obtained using the sensor by ensuring that the chemical reaction is localized at a site remote from the electrode used to electrochemically react the electroactive product(s).

The site of the chemical reaction is sufficiently removed from the electrode such that the mass transfer of the electroactive species from the chemical reaction site to the electrode effectively controls the current flowing at the

electrode at any time. This arrangement ensures a substantially linear electroactive species concentration gradient between the chemical reaction site and the electrode. The concentration of the electroactive species is maintained at effectively zero at the electrode by the electrochemical reaction taking place there. The time course of the magnitude of this concentration gradient will therefore be substantially determined only by the time course of the concentration of the electroactive specie(s) at the chemical reaction site and the diffusion coefficient(s) of the electroactive reaction product(s) in the liquid medium. Since the current flowing at the electrode is proportional to the concentration gradient of the electroactive specie(s) at the electrode, the time course of this current will reflect the time course of the chemical reaction occurring at the remote site. This allows the current measured at the electrode (or charge passed if the current is integrated) to be a used as a convenient measure of the rate and extent of the chemical reaction taking place.

An example of a suitable method for ensuring that the chemical reaction is remote from the working electrode is to immobilize one or more of the reaction components on a solid surface remote from the electrode. The reaction component(s) can be immobilized by incorporating them in a polymeric matrix that is dried on or otherwise attached to the solid surface. The reaction component(s) can also be tethered directly to the solid surface either by chemical or physical bonding. Alternatively one or more of the reaction components can simply be dried onto the solid surface without special immobilization means. In this situation one or more of the reaction components is sufficiently low in mobility, in the liquid matrix filling the electrochemical cell, that it does not migrate substantially from the position where it was dried during the time period that the electrochemical current can be usefully monitored to perform the required measurement. In this context substantial migration means that the slowest moving component required for the chemical reaction approaches closely enough to the working electrode that Cottrell type depletion kinetics begin to effect the time course of the current flowing at the electrode.

The range of separation distance between the chemical reaction site and the working electrode in preferred embodiments is desirably less than about 1 cm, preferably less than 5 mm, more preferably between 5, 10, 50, 100, 200, 500 microns and 5 mm, more preferably between 5, 10, 50, 100, 200 and 500 microns, and most preferably between 5, 10, 50, 100 and 200 microns.

As well as the working electrode, at least a counter electrode in contact with the liquid sample is provided to complete the electrochemical circuit. Optionally the counter electrode can function as a combined counter/reference electrode or a separate reference electrode can be provided. In a preferred embodiment, the working electrode and counter electrode are desirably spaced apart at a distance greater than about 300 microns, preferably at a distance greater than about 500 microns, more preferably at a distance between about 500 microns and 10 mm, more preferably at a distance between about 500 microns and 1, 2, 5 mm, and most preferably between 1 mm and 2, 5, 10 mm.

The working electrode is constructed of materials that do not react chemically with any component with which it will come into contact during use to an extent that interferes with the current response of the electrode. If the working electrode is to be used as an anode then examples of suitable materials are platinum, palladium, carbon,

carbon in combination with inert binders, iridium, indium oxide, tin oxide, mixtures of indium and tin oxide. If the working electrode is to be used as a cathode then in addition to the material listed above other suitable materials are steel, stainless steel, copper, nickel, silver and chromium.

Examples of materials suitable for the counter electrode are platinum, palladium, carbon, carbon in combination with inert binders, iridium, indium oxide, tin oxide, mixture of indium and tin oxide, steel, stainless steel, copper, nickel, chromium, silver and silver coated with a substantially insoluble silver salt such as silver chloride, silver bromide, silver iodide, silver ferrocyanide, silver ferricyanide.

The site of the chemical reaction can be localized on a bare wall or on the counter electrode, remote from the working electrode. The site of the chemical reaction can be on the same plane as the working electrode or more preferably in a plane facing and substantially parallel to the working electrode.

A sensor suitable for use with certain embodiments includes a working electrode and a counter electrode which are disposed on an electrically insulating substrate. On a second substrate is disposed a layer of chemical reactants, where at least one of the reactants is substantially immobilized on the substrate. In use, the space between walls of the sensor is filled with a liquid containing a substance which is capable of reacting with the reagents to produce at least one electroactive species. The products of the chemical reaction diffuse towards the working electrode where the electroactive specie(s) are electrochemically reacted to produce a current. The magnitude of the current or the charge passed at a particular time, or the time course of the current or charge passed can then be used to obtain a measure of the rate or extent of the chemical reaction occurring at the reactant layer.

In another embodiment of the sensor, the reactants are disposed on the counter electrode which is disposed on an electrically resistive substrate. In this embodiment the materials of construction of the counter electrode are inert to reaction with any of the components of the reactants disposed on the electrode.

The method of obtaining an electrochemical measurement described above may be applied to any suitable electrochemical system, including electrochemical immunoassay systems. An example of the method as applied to a typical, albeit non-immunoassay, electrochemical system is measuring glucose in whole blood using the enzyme PQQ dependent glucose dehydrogenase (GDHpqq) and a redox mediator. In this reaction glucose in the blood reacts with GDHpqq to form gluconic acid. In the process, the PQQ in the enzyme is reduced. A mediator, such as potassium ferricyanide, then oxidizes the PQQ in the enzyme and forms ferrocyanide. The enzyme in the oxidized form can then react with further glucose. The net effect of this reaction is to produce two ferrocyanide molecules for each glucose molecule reacted. Ferrocyanide is an electroactive species, and so can be oxidized at an electrode to produce a current. Other suitable enzymes for this reaction are glucose oxidase (GOD) or NAD dependent glucose dehydrogenase. For other reactions, lactate dehydrogenase and alcohol dehydrogenase may be used. Other suitable redox mediators include ferrocinium, osmium complexes with bipyridine, and benzophenone.

The reaction of glucose in whole blood with the enzyme can be slow, taking up to a few minutes to go to completion. Also, the higher the haematocrit of the blood sample, the slower the reaction. The haematocrit of the blood is the volume fraction of red cells in the whole blood sample. For example, a solution containing 50 mg/ml

GDHpqq, 0.9 M potassium ferricyanide and 50 mM buffer at pH 6.5 was deposited on the counter electrode and the water removed to leave a dried reactant layer. In this layer the GDHpqq is large enough to be effectively immobilized on the counter electrode, whereas the ferricyanide can mix more evenly throughout the liquid in the electrochemical cell. The blood sample was introduced into the cell and a potential of +300 mV immediately applied between the working electrode and the counter electrode. Although a potential of +300 mV is most preferred for oxidizing ferrocyanide, the potential is desirably between +40 mV and +600 mV, preferably between +50 mV and +500 mV, and more preferably between +200 mV and +400 mV. In the cell, the working electrode consisted of a layer of gold sputtered onto a polyester substrate and the counter electrode consisted of a layer of palladium sputtered onto a polyester substrate.

Current traces were recorded for blood samples of different haematocrits, showing a faster rate of reaction in lower haematocrit blood, i.e., 20%, 42%, and 65% haematocrit in blood. The glucose level in each blood sample was approximately the same, namely 5.4 mM for the 65% haematocrit sample, 5.5 mM for the 42% haematocrit sample, and 6.0 mM for the 20% haematocrit sample.

The current measured can be approximately given by the equation:

$$i = -FADC/L$$

where i is the current, F is Faraday's constant (96486.7 C/mole), A is the electrode area, D is the diffusion coefficient of the ferrocyanide in the sample, C is the concentration of ferrocyanide at the reaction site and L is the distance between the reaction site and the electrode. The reaction rate, given by the rate of change of C with time is therefore given by:

$$dC/dt = -(L/FAD)di/dt.$$

For the reactions discussed above, between 6 and 8 seconds for the 20%, 42%, and 65% haematocrit samples, the average di/dt was 3.82, 2.14 and 1.32 microamps/second, respectively. The diffusion coefficients of ferrocyanide for these samples were 2.0×10^{-6}, 1.7×10^{-6} and 1.4×10^{-6} cm^2/sec for 20%, 42%, and 65% haematocrit samples, respectively. The electrode area was 0.1238 cm^2 and L was 125 microns. These values yield reaction rates of 2.0, 1.3, and 0.99 mM/second for the 20%, 42%, and 65% haematocrit samples, respectively.

The method as described above for measuring the reaction of glucose in blood may be suitably modified to apply to other electrochemical systems, including immunoassay systems, such as antigen determination, as will be appreciated by one skilled in the art.

The above description discloses several methods and materials of the present invention. This invention is susceptible to modifications in the methods and materials, as well as alterations in the fabrication methods and equipment. Such modifications will become apparent to those skilled in the art from a consideration of this disclosure or practice of the invention disclosed herein. Consequently, it is not intended that this invention be limited to the specific embodiments disclosed herein, but that it cover all modifications and alternatives coming within the true scope and spirit of the invention as embodied in the attached claims.

WHAT IS CLAIMED IS:

1. A disposable device for use in detecting a target antigen in a fluid sample having a pH, the device comprising a reaction chamber having an internal surface, a proximal end, and a distal end; an immobilized antibody fixed within the reaction chamber, the antibody being capable of binding to the target antigen; a reporter complex present within the reaction chamber, the complex comprising a probe, the reporter complex being capable of mixing with the sample; a detection chamber having a wall, an internal surface, a distal end and a proximal end; a sample ingress at the distal end of the reaction chamber; and a sample passageway between the distal end of the reaction chamber and the proximal end of the detection chamber.

2. The device of claim 1, further comprising an agent contained within the reaction chamber and capable of preventing non-specific binding of proteins to the reaction chamber internal surface.

3. The device of claim 2, wherein the agent is selected from the group consisting of a surfactant and a blocking protein.

4. The device of claim 3, wherein the blocking protein is bovine serum albumin.

5. The device of claim 1, wherein the reporter complex further comprises a second antigen capable of competing with the target antigen for binding to the immobilized antibody, or a second antibody capable of binding to the target antigen.

6. The device of claim 1, wherein the probe is selected from the group consisting of chromophores and fluorophores.

7. The device of claim 1, wherein the probe comprises an enzyme.

8. The device of claim 7, wherein the enzyme comprises glucose oxidase.

9. The device of claim 7, wherein the enzyme comprises glucose dehydrogenase.

10. The device of claim 7, the detection chamber further comprising an enzyme substrate.

11. The device of claim 7, wherein the enzyme substrate is an oxidizable substrate.

12. The device of claim 11, wherein the oxidizable substrate is selected from the group consisting of galactose and acetic acid.

13. The device of claim 11, wherein the enzyme substrate comprises glucose.

14. The device of claim 7, the detection chamber further comprising a mediator.

15. The device of claim 14, wherein the mediator is selected from the group consisting of dichlorophenolindophenol and complexes between transition metals and nitrogen-containing heteroatomic species.

16. The device of claim 14, wherein the mediator comprises ferricyanide.

17. The device of claim 7, the device further comprising a buffer capable of adjusting the pH of the sample.

18. The device of claim 17, wherein the buffer is a substance selected from the group consisting of comprises phosphate and citrate.

19. The device of claim 1, wherein the detection chamber comprises at least two electrodes.

20. The device of claim 19, wherein the electrodes comprise a material selected from the group consisting of palladium, platinum, gold, iridium, carbon, carbon mixed with binder, indium oxide, tin oxide, and mixtures thereof.

21. The device of claim 1, wherein the detection chamber wall is transparent to a radiation emitted or absorbed by the probe, wherein the radiation is indicative of the presence or absence of the reporter complex in the detection chamber.

22. The device of claim 1, further comprising a detector capable of detecting a condition wherein the reaction chamber is substantially filled.

23. The device of claim 1, further comprising a piercing means capable of forming a detection chamber vent in the distal end of the detection chamber.

24. The device of claim 1, further comprising a reaction chamber vent at the distal end of the reaction chamber.

25. A method of manufacture of a disposable device for use in detecting a target antigen in a fluid sample having a pH, the method comprising the steps of:

forming a first aperture extending through a first sheet of material having a proximal end and a distal end, the first aperture defining a reaction chamber side wall, a detection chamber side wall and a first sample passageway between the reaction chamber distal end and the detection chamber proximal end;

mounting a first layer to a first side of the first sheet and extending over the aperture to define a first reaction chamber end wall and a first detection chamber end wall;

mounting a second layer to a second side of the first sheet and extending over the aperture to define a second reaction chamber end wall and a second detection chamber end wall in substantial overlying registration with the first layer, whereby the sheet and layers form a strip having a plurality of exterior surfaces;

forming a second passageway extending through an exterior surface of the strip and into the reaction chamber at the reaction chamber distal end, the second passageway defining a reaction chamber vent;

forming a third passageway extending through the an exterior surface of the strip and into the reaction chamber at the reaction chamber proximal end, the third passageway defining a sample ingress;

immobilizing an antibody within the reaction chamber; and

placing a reporter complex in the reaction chamber, the complex comprising a probe.

26. The method of claim 25, wherein the first sheet, the first layer and the second layer comprise an electrically resistive material, the first layer comprises a first electrode wherein the first electrode faces the first side of the first sheet, and the second layer comprises a second electrode wherein the second electrode faces the second side of the sheet.

27. The method of claim 26, wherein the second electrode is mounted in opposing relationship a distance of less than about 500 microns from the first electrode.

28. The method of claim 26, wherein the second electrode is mounted in opposing relationship a distance of less than about 150 microns from the first electrode.

29. The method of claim 26, wherein the second electrode is mounted in opposing relationship a distance of less than about 150 microns and greater than about 50 microns from the first electrode.

30. The method of claim 25, wherein at least one of the layers is transparent to a wavelength of radiation selected from the group consisting of infrared radiation, visible light, and ultraviolet radiation.

31. The method of claim 25, further comprising the step of:

providing an enzyme substrate and a mediator, wherein the enzyme substrate and the mediator are contained within the detection chamber, wherein the probe is an enzyme, and wherein the mediator is capable of mediating a reaction between the enzyme and the electrode, to indicate the occurrence of an electrochemical reaction.

32. A method of manufacture of a disposable device for use in detecting a target antigen in a fluid sample having a pH, the method comprising:

forming a first aperture extending through a first sheet of electrically resistive material having a proximal end and a distal end, the first aperture having a first aperture reaction chamber part and a first aperture detection chamber part and defining a first portion of a reaction chamber side wall, a detection chamber side wall and a sample passageway between the reaction chamber distal end and the detection chamber proximal end;

forming a second aperture extending through a second sheet of electrically resistive material having a proximal end and a distal end, the second aperture defining a second portion of the reaction chamber side wall;

forming a third aperture extending through a third sheet of electrically resistive material having a proximal end and a distal end, the third aperture defining a third portion of the reaction chamber side wall;

mounting a first side of the second sheet to a first side of the first sheet, the second sheet extending over the first aperture detection chamber part whereby to define a first detection chamber end wall, the second portion of the reaction chamber side wall in substantial registration with the first portion of the reaction chamber side wall;

mounting a first side of the third sheet to a second side of the first sheet, the third sheet extending over the first aperture detection chamber part whereby to define a second detection chamber end wall, the third portion of the reaction chamber side wall in substantial registration with the first portion of the reaction chamber side wall;

mounting a first layer to a second side of the second sheet and extending over the second aperture to define a first reaction chamber end wall;

mounting a second layer to a second side of the third sheet and extending over the third aperture to define a second reaction chamber end wall in substantial overlying registration with the first thin layer, whereby the sheets and layers form a strip having a plurality of exterior surfaces;

forming a second passageway extending through the outside of the strip and into the reaction chamber at the reaction chamber distal end, the second passageway defining a reaction chamber vent;

forming a third passageway extending through the outside of the strip and into the reaction chamber at the reaction chamber proximal end, the third passageway defining a sample ingress;

immobilizing an antibody within the reaction chamber; and

placing a reporter complex in the reaction chamber, the reporter complex comprising a probe.

33. A method for determining a presence or an absence of a target antigen in a fluid sample, the method comprising:

providing the device of claim 1, wherein the reporter complex further comprises a second antigen capable of competing with the target antigen for binding to the immobilized antibody;

contacting a fluid sample with the sample ingress;

substantially filling the reaction chamber with the fluid sample by allowing the sample to flow from the sample ingress toward the reaction chamber;

allowing a predetermined time to lapse, the time being sufficient for substantially all reporter complex to bind to the immobilized antibody in the absence of antigen in the sample;

substantially filling the detection chamber with the fluid sample by allowing the sample to flow from the reaction chamber through the sample passageway toward the detection chamber;

detecting a presence or an absence of the antigen-probe complex within the detection chamber, the presence or absence of the antigen-probe complex being indicative of a presence or an absence of the antigen in the sample.

34. A method of manufacture of a disposable device for use in detecting a target antigen in a fluid sample having a pH, the device having a plurality of exterior surfaces, the method comprising:

forming a first aperture extending through a first sheet of electrically resistive material, the first aperture having a detection chamber part and defining a detection chamber side wall, the detection chamber having a proximal end and a distal end;

mounting a first layer to a first side of the first sheet and extending over the aperture to define a first detection chamber end wall;

mounting a second layer to a second side of the first sheet and extending over the aperture to define a second detection chamber end wall in substantial overlying registration with the first layer, whereby the sheet and layers form a strip;

forming a second aperture extending through the strip, the strip having a proximal end and a distal end, the second aperture having a reaction chamber part, the reaction chamber having a distal end and a proximal end, and the second aperture defining a reaction chamber side wall and a sample passageway between the reaction chamber distal end and the detection chamber proximal end;

mounting a first side of a third layer to a first side of the strip, the third extending over the second aperture reaction chamber part to define a first reaction chamber end wall;

mounting a first side of a fourth layer to a second side of the strip, the fourth layer extending over the second aperture reaction chamber part whereby to define a second reaction chamber end wall in substantial registration with the first reaction chamber end wall;

forming a third aperture extending through a surface of the device and into the reaction chamber at the reaction chamber distal end, the third aperture defining a reaction chamber vent;

forming a fourth aperture extending through a surface of the device and into the reaction chamber at the reaction chamber proximal end, the fourth aperture defining a sample ingress;

immobilizing an antibody within the reaction chamber; and

placing a reporter complex in the reaction chamber, the reporter complex comprising a probe.

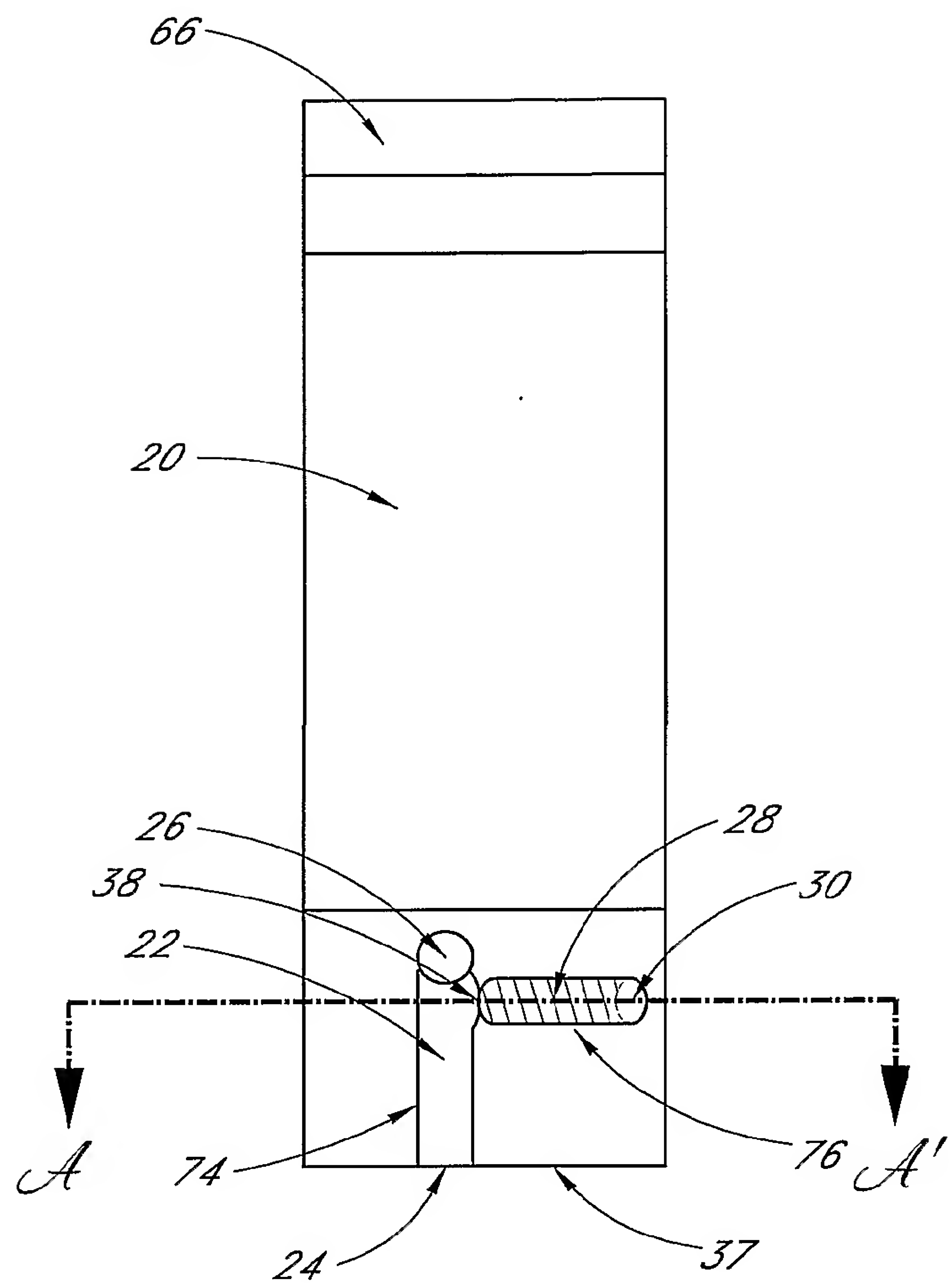

Fig. 1

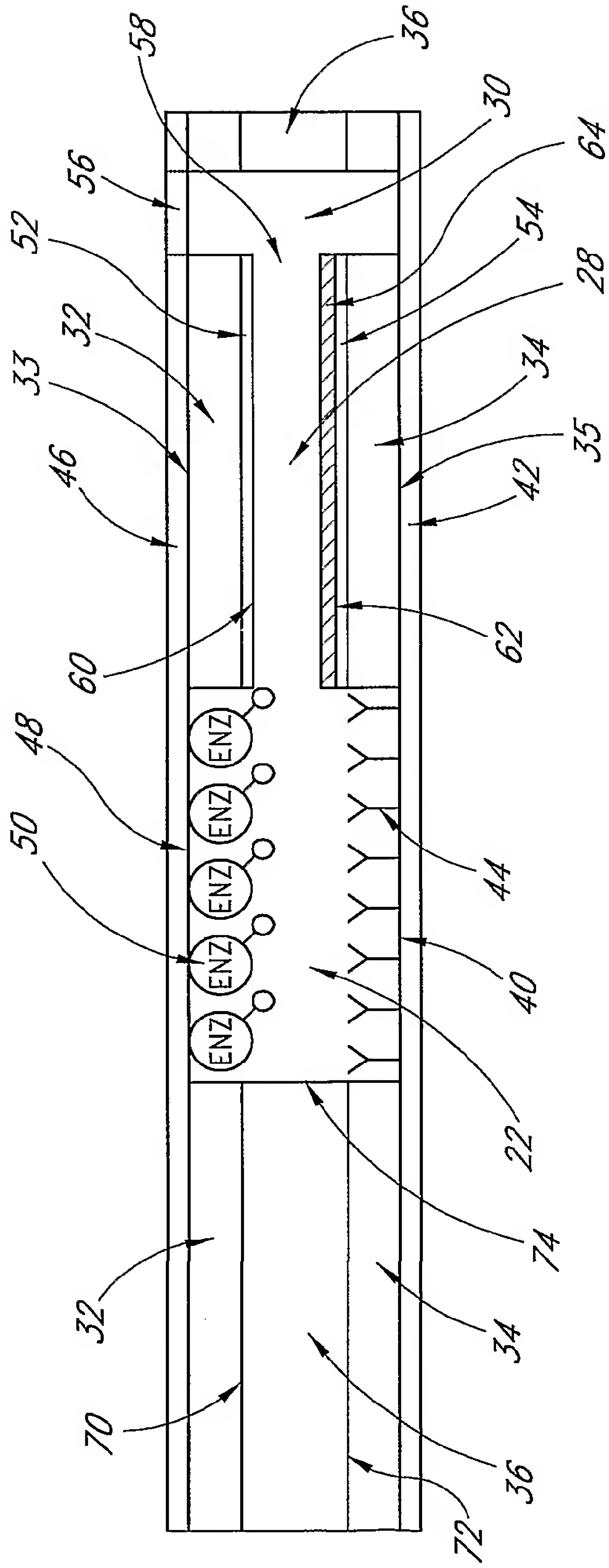
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Fig. 2